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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/006,922	12/04/2001	Sergey A. Lukyanov	CLON-035CIP	9351

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EXAMINER
ROBINSON, HOPE A

ART UNIT	PAPER NUMBER
1656	

DATE MAILED: 08/02/2006

Please find below and/or attached an Office communication concerning this application or proceeding.

Advisory Action Before the Filing of an Appeal Brief	Application No. 10/006,922	Applicant(s) LUKYANOV ET AL.	
	Examiner Hope A. Robinson	Art Unit 1656	

--The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

THE REPLY FILED 12 July 2006 FAILS TO PLACE THIS APPLICATION IN CONDITION FOR ALLOWANCE.

1. ☐ The reply was filed after a final rejection, but prior to or on the same day as filing a Notice of Appeal. To avoid abandonment of this application, applicant must timely file one of the following replies: (1) an amendment, affidavit, or other evidence, which places the application in condition for allowance; (2) a Notice of Appeal (with appeal fee) in compliance with 37 CFR 41.31; or (3) a Request for Continued Examination (RCE) in compliance with 37 CFR 1.114. The reply must be filed within one of the following time periods:
   a) ☒ The period for reply expires 2 months from the mailing date of the final rejection.
   b) ☐ The period for reply expires on: (1) the mailing date of this Advisory Action, or (2) the date set forth in the final rejection, whichever is later. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the mailing date of the final rejection.

   Examiner Note: If box 1 is checked, check either box (a) or (b). ONLY CHECK BOX (b) WHEN THE FIRST REPLY WAS FILED WITHIN TWO MONTHS OF THE FINAL REJECTION. See MPEP 706.07(f).

Extensions of time may be obtained under 37 CFR 1.136(a). The date on which the petition under 37 CFR 1.136(a) and the appropriate extension fee have been filed is the date for purposes of determining the period of extension and the corresponding amount of the fee. The appropriate extension fee under 37 CFR 1.17(a) is calculated from: (1) the expiration date of the shortened statutory period for reply originally set in the final Office action; or (2) as set forth in (b) above, if checked. Any reply received by the Office later than three months after the mailing date of the final rejection, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

NOTICE OF APPEAL

2. ☐ The Notice of Appeal was filed on _____. A brief in compliance with 37 CFR 41.37 must be filed within two months of the date of filing the Notice of Appeal (37 CFR 41.37(a)), or any extension thereof (37 CFR 41.37(e)), to avoid dismissal of the appeal. Since a Notice of Appeal has been filed, any reply must be filed within the time period set forth in 37 CFR 41.37(a).

AMENDMENTS

3. ☐ The proposed amendment(s) filed after a final rejection, but prior to the date of filing a brief, will not be entered because
   (a) ☐ They raise new issues that would require further consideration and/or search (see NOTE below);
   (b) ☐ They raise the issue of new matter (see NOTE below);
   (c) ☐ They are not deemed to place the application in better form for appeal by materially reducing or simplifying the issues for appeal; and/or
   (d) ☐ They present additional claims without canceling a corresponding number of finally rejected claims.

   NOTE: _____. (See 37 CFR 1.116 and 41.33(a)).
4. ☐ The amendments are not in compliance with 37 CFR 1.121. See attached Notice of Non-Compliant Amendment (PTOL-324).
5. ☐ Applicant's reply has overcome the following rejection(s): _____.
6. ☐ Newly proposed or amended claim(s) _____ would be allowable if submitted in a separate, timely filed amendment canceling the non-allowable claim(s).
7. ☒ For purposes of appeal, the proposed amendment(s): a) ☐ will not be entered, or b) ☒ will be entered and an explanation of how the new or amended claims would be rejected is provided below or appended.
   The status of the claim(s) is (or will be) as follows:
   Claim(s) allowed: _____.
   Claim(s) objected to: _____.
   Claim(s) rejected: *1-13,18-23,27 and 31-62*.
   Claim(s) withdrawn from consideration: _____.

AFFIDAVIT OR OTHER EVIDENCE

8. ☐ The affidavit or other evidence filed after a final action, but before or on the date of filing a Notice of Appeal will not be entered because applicant failed to provide a showing of good and sufficient reasons why the affidavit or other evidence is necessary and was not earlier presented. See 37 CFR 1.116(e).
9. ☐ The affidavit or other evidence filed after the date of filing a Notice of Appeal, but prior to the date of filing a brief, will not be entered because the affidavit or other evidence failed to overcome all rejections under appeal and/or appellant fails to provide a showing a good and sufficient reasons why it is necessary and was not earlier presented. See 37 CFR 41.33(d)(1).
10. ☐ The affidavit or other evidence is entered. An explanation of the status of the claims after entry is below or attached.

REQUEST FOR RECONSIDERATION/OTHER

11. ☒ The request for reconsideration has been considered but does NOT place the application in condition for allowance because: See the attached sheets.
12. ☐ Note the attached Information Disclosure Statement(s). (PTO/SB/08 or PTO-1449) Paper No(s). _____
13. ☐ Other: _____.

DETAILED ACTION

Application Status

1. Applicant's response to the Final Office Action mailed May 25, 2006 on July 12, 2006 is acknowledged.

Claim Disposition

2. Claims 1-13, 18-23, 27 and 31-62 are pending and are under examination.

Maintained-Claim Rejections - 35 USC э 112

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

3. Claims 1-13, 18-23, 27 and 31-62 remain rejected under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

The claimed invention is directed to a nucleic acid present in other than its natural environment that encodes a chromo or fluorescent protein wherein said protein has a sequence identity of at least 70%, 75%, 80% or 85% sequence identity to SEQ ID NO:12. This represents

a partial structure and a skilled artisan would not be able to envision the detailed chemical structure of the genus of proteins encompassed the claims. There is no indication in the claims or specification as to where in the claims modifications will occur. Thus, the instant specification fails to provide adequate description for the large genus of proteins encompassed in the claims. The claims encompass mutations other than point mutations or single deletions, which have not been described. The specification fails to provide a representative number of species for the claimed genus to show that applicant was in possession of the claimed genus. A representative number of species means that the species, which are adequately described, are representative of the entire genus. In addition, the claims recite that said nucleic acid encodes a protein that has one or more amino acid substitutions selected from amino acid substitutions at positions 2, 5, 9, 105 or 197 as compared to SEQ ID NO:12. The language in the claim is open as "has" is interpreted as "comprising" and the phrase "one or more amino acid substitutions" does not place a limit on whether position 2 for example, will have 1 or 5 or 10 or 15 substitutions or describe the possible combinations. Additionally, the claimed invention is directed to "one or more amino acid substitutions selected from R2A, K5E, K9T, V105A and S197T". A genus of mutations are encompassed in this claim language, for example the following combinations can occur and a lot more: (a)R2A, K5E, K9T, V105A, S197T; (b)R2A, K5E ; (c)R2A, K9T; (d)R2A,V105A; (e)R2A, S197T; (f) K5E, K9T; (g)K5E, V105A; (h)K5E, S197T; (i)K9T, V105A; K9T, S197T; (j)V105A, S197T; (k)R2A, K5E, K9T; (l)R2A, K5E, V105A; (m)R2A, K5E, S197T; (n)K5E, V105A, K9T. The instant specification on page 6 discloses that "[T]he proteins of interest are proteins that are colored and/or fluorescent, where this feature arises from the interaction of two or more residues of the protein". The specification does not provide

adequate written description of which two or more residues results in the desired effect and as demonstrated above the claimed invention encompasses a genus of mutants based on the recited "one or more substitutions". A skilled artisan would have to determine which two point mutations or combination of mutations will produce the desired effect/interaction. Therefore, the claimed invention lacks adequate written description for the genus of proteins encompassed in the claims.

Further, *Vas-Cath Inc. v. Mahurkar, 935 F.2d 1555, 1563-64, 19 USPQ2d 1111, 1117 (Fed. Cir.1991)*, states that "applicant must convey with reasonable clarity to those skilled in the art that, as of the filing date sought, he or she was in *possession of the invention*. The invention is, for purposes of the 'written description' inquiry, *whatever is now claimed*" (See page 1117). The specification does not "clearly allow persons of ordinary skill in the art to recognize that [he or she] invented what is claimed" (See *Vas-Cath* at page 1116). The skilled artisan cannot envision the detailed chemical structure of the encompassed genus of proteins encoded the claimed nucleic acid, and therefore, conception is not achieved until reduction to practice has occurred, regardless of the complexity or simplicity of the method of isolation. Adequate written description requires more than a mere statement that it is part of the invention and reference to a potential method of isolating it. The compound itself is required. *See Fiers v. Revel, 25 USPQ2d 1601 at 1606 (CAFC 1993).*

Therefore, for all these reasons the specification lacks adequate written description, and one of skill in the art cannot reasonably conclude that the applicant had possession of the claimed invention at the time the instant application was filed.

4. Claims 1-13, 18-23, 27 and 31-62 remain rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for the nucleic acid set forth in SEQ ID NO: 11 that encodes the protein set forth in SEQ ID NO:12 and specific point mutations exemplified in the specification, does not reasonably provide enablement for any fragment thereof or a transgenic organism or progeny thereof. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make the invention commensurate in scope with these claims. The enablement requirement refers to the requirement that the specification describe how to make and how to use the invention. There are many factors to be considered when determining whether there is sufficient evidence to support a determination that a disclosure does not satisfy the enablement requirement and whether any necessary experimentation is undue. These factors include, but are not limited to: Quantity of Experimentation Necessary; Amount of direction or guidance presented; Presence or absence of working examples; Nature of the Invention; State of the prior art and Relative skill of those in the art; Predictability or unpredictability of the art and Breadth of the claims (see *In re Wands, 858 F.2d at 737, 8 USPQ2d at1404 (Fed. Cir. 1988*). The factors most relevant to the instant invention are discussed below.

The amount of experimentation required to practice the claimed invention is undue as the claims encompass an unspecified amount of fragments. The claimed invention is directed to a nucleic acid present in other than its natural environment encoding a protein having a sequence identity of at least about 70-85% to SEQ ID NO:12. No guidance is provided in the instant specification as to any special feature/characteristics or the structure of all the possible fragments encompassed by the claims. There are no indicia as to how much modifications can be tolerated

in the wild type structures. The claims encompass mutations that are not limited to a single point mutation and encompass a large variable genus of proteins. In addition, newly submitted claims 53-62 recite that said nucleic acid encodes a protein that has one or more amino acid substitutions selected from amino acid substitutions at positions 2, 5, 9, 105 or 197 as compared to SEQ ID NO:12 and the claims are also directed to "one or more amino acid substitutions selected from R2A, K5E, K9T, V105A and S197T compared to SEQ ID NO:12". The language in the claim is open as "has" is interpreted as "comprising" and the phrase "one or more amino acid substitutions" does not place a limit on whether position 2 for example, will have 1 or 5 or 10 or 15 substitutions or describe the possible combinations (see for example claim 53). Furthermore, a genus of mutations are encompassed in the claim language (see claim 54), for example the following combinations can occur and a lot more: (a)R2A, K5E, K9T, V105A, S197T; (b)R2A, K5E ; (c)R2A, K9T; (d)R2A,V105A; (e)R2A, S197T; (f) K5E, K9T; (g)K5E, V105A; (h)K5E, S197T; (i)K9T, V105A; K9T, S197T; (j)V105A, S197T; (k)R2A, K5E, K9T; (l)R2A, K5E, V105A; (m)R2A, K5E, S197T; (n)K5E, V105A, K9T. Note that the instant specification on page 6 disclose that "[T]he proteins of interest are proteins that are colored and/or fluorescent, where this feature arises from the interaction of two or more residues of the protein". The specification does not provide adequate guidance as to which two or more residues results in the desired effect and as demonstrated above the claimed invention encompasses a genus of mutants based on the recited "one or more substitutions". No correlation is made between structure and function of the encoded protein. A skilled artisan would have to perform undue experimentation to construct all the claimed fragments absent guidance.

The instant specification does not demonstrate or provide guidance as to what the structure of the protein will be once modified based on the changes contemplated in the claims and the instant specification (i.e. 70-85% sequence identity). Based on vast amount of modifications encompassed in the claims said nucleic acid might encode a protein that is non-functional or different. Additionally, there is no data provided demonstrative of a particular portion of the structure that must be conserved. One skilled in the art would have to engage in undue experimentation to construct for example, a fragment thereof and then produce from this a chromo protein or fluorescent protein that maintains the recited properties. Due to the large quantity of experimentation necessary to generate the infinite number of fragments recited in the claims and possibly screen same for activity/desired properties and the lack of guidance/direction provided in the instant specification, this is merely an invitation to the skilled artisan to use the current invention as a starting point for further experimentation. Thus, undue experimentation would be required for a skilled artisan to make and/or use the claimed invention commensurate in scope with the claims.

Predictability of which potential changes can be tolerated in a protein's amino acid sequence and obtain the desired activity requires a knowledge of and guidance with regard to which amino acids in the protein's sequence, if any, are tolerant of modification and which are conserved and detailed knowledge of the ways in which the protein's structure relates to its function. In addition, one skilled in the art would expect any tolerance to modification for a given protein to diminish with each further and additional modification, for example, multiple substitutions. In this case, the necessary guidance has not been provided in the specification. Therefore, while it is known in the art that many amino acid substitutions are possible in any

given protein, the positions within the protein's sequence where such amino acid substitutions can be made with a reasonable expectation of success are limited, as certain positions in the sequence are critical to the protein's structure/function relationship. It is also known in the art that a single nucleotide or amino acid change or mutation can destroy the function of the biomolecule in many cases. For example, various sites or regions directly involved in binding activity and in providing the correct three-dimensional spatial orientation of binding and active sites can be affected (see Wells, Biochemistry, vol. 29, pages 8509-8517, 1990). It is in no way predictable that randomly selected mutations, such as deletions, substitutions, additions, etc., in the disclosed sequences would result in a protein having activity/properties comparable to the one disclosed. As plural substitutions for example are introduced, their interactions with each other and their effects on the structure and function of the protein is unpredictable. It is noted that the instant specification provides for example point mutations, however, the claims encompass plural substitutions, which are not exemplified, nor are there examples of all the possible mutant sequences. Thus, the skilled artisan would recognize the high degree of unpredictability that all the fragments/mutants encompassed in the claims would retain the recited properties.

The state of the prior art provides evidence for the high degree of unpredictability as stated above. For example, Heim et al. (PNAS, vol. 91, pages 12501-04, 1994) disclose that a mutated DNA was sequenced and found to contain five amino acid substitutions, only one of which was found to be critical, Tyr66His, in the center of the chromophore. Heim et al. also disclose further site directed mutagenesis and noted that there was tolerance of the substitutions

made, however, some mutants were weakly fluorescent (page 12504). Therefore, amino acid substitutions are critical to the protein's structure/function relationship.

The specification lacks adequate guidance/direction to enable a skilled artisan to practice the claimed invention commensurate in scope with the claims. Furthermore, while recombinant and mutagenesis techniques are known in the art, it is not routine in the art to screen large numbers of fragments where the expectation of obtaining similar activity is unpredictable based on the instant disclosure. Moreover, the amino acid sequence of a protein determines its structural and functional properties, and predictability of what mutations can be tolerated in a protein's sequence and result in certain activity/property, which is very complex, and well outside the realm of routine experimentation, because accurate predictions of a protein's function from mere sequence data are limited, therefore, the general knowledge and skill in the art is not sufficient, thus the specification needs to provide an enabling disclosure.

The working examples provided do not rectify the missing information in the instant specification pertaining to the claimed mutants/fragments as the claims encompass mutants/fragments not described in the instant specification. Thus, one of skill in the art would have to engage in undue experimentation to construct the mutants/fragments of the claimed invention and examine the same for function/the specific properties.

The specification does not provide support for the broad scope of the claims, which encompass an unspecified amount of fragments. The claims broadly read on a whole organism and a transgenic cell or organism, for example a human for which no support is provided in the instant specification. The issue in this case is the breath of the claims in light of the predictability of the art as determined by the number of working examples, the skill level artisan and the

guidance presented in the instant specification and the prior art of record. This make and test position is inconsistent with the decisions of *In re Fisher, 427 F.2d 833, 839, 166 USPQ 18, 24 (CCPA 1970)* where it is stated that "...scope of claims must bear a reasonable correlation to scope of enablement provided by the specification to persons of ordinary skill in the art...". Without sufficient guidance, determination of having the desired biological characteristics is unpredictable and the experimentation left to those skilled in the art is unnecessarily and improperly extensive and undue. See *In re Wands, 858 F.2d at 737, 8 USPQ2d at 1404 (Fed. Cir. 1988)*. Therefore, the instant specification to be enabling need to provide direction/guidance regarding whether the structure of the chromo or fluorescent fragment/mutant can tolerate the modifications encompassed by claims and still possess the desired properties or whether a protein that does not have the desired properties may result. Absent sufficient guidance/direction one of skill in the art would not be able to practice the claimed invention commensurate in scope with the claims.

Thus, for all these reasons, the specification is not considered to be enabling for one skilled in the art to make and use the claimed invention as the amount of experimentation required is undue, due to the broad scope of the claims, the lack of guidance and insufficient working examples provided in the specification and the high degree of unpredictability as evidenced by the state of the prior art, attempting to construct and test mutants of the claimed invention would constitute undue experimentation. Making and testing the infinite number of possible fragments to find one that has the desired properties as described is undue experimentation. Therefore, applicants have not provided sufficient guidance to enable one of

skill in the art to make and use the claimed invention in a manner that reasonably correlates with the scope of the claims, to be considered enabling.

Withdrawn-Claim Rejections - 35 USC ∋ 112

5. Previous rejection to claims under 35 U.S.C. 112, second paragraph, is withdrawn by virtue of submission of an amendment.

Response to Arguments:

6. Applicant's response filed on July 12, 2006 has been considered, however is not persuasive. Note that the rejections of record remain under 35 U.S.C. 112, first paragraph for the reasons of record and herein.

On pages 10-12 Applicant addresses the rejection under 35 U.S.C. 112, first paragraph written description. Applicant on page 11 state that the specification provides at least ten different variants of SEQ ID NO:12 and indicate that SEQ ID NOS:14, 28, 42, 6 and 8 are all at least 70% identical to SEQ ID NO:12. Applicant provided an alignment also labeled Exhibit A and indicated that conserved regions were highlighted. All arguments and exhibits submitted have been considered in full, however, were not found to be persuasive. Firstly, Exhibit A provided by applicant did not demonstrate the stated 70% identity of SEQ ID NO:12 with SEQ ID NOS:14, 28, 42, 6 and 8, therefore an alignment was performed to see if Applicant's statements were accurate. Note that SEQ ID NO:42 is 46.1% identical to SEQ ID NO:12; SEQ ID NO:14 is 45.8% identical to SEQ ID NO:12; SEQ ID NO:28 is 40.7% identical to SEQ ID NO:12; SEQ ID NO:6 is 39.4% identical to SEQ ID NO:12; and SEQ ID NO:8 is 39.1%

identical to SEQ ID NO:12 (see the attached alignments). In addition, the conserved regions pointed to by applicant in exhibit A consisted of approximately 1-6 residue matches, thus, the sequences do not have long contiguous stretches of residues in common. Further, Applicant opines that at least 10 different variants of SEQ ID NO:12 is reported in the specification, this argument is not persuasive in the face of all the variability contemplated in the structure of the claimed protein. Note that SEQ ID NO:12 has 225 residues for which 30% variability is allowed in for example claim 1 of the instant application which is equivalent to changing 68 residues any where in the sequence. Thus, 68 residues can be varied with any of the 20 naturally occurring amino acid (which represents 68 to the power of 20 or 68 $x10^{20}$), thus Applicant's 10 variants is hardly representative of the enormous amount of variability encompassed in the claims. The instant claims are not limited to Applicant's purported 10 variants. Therefore, the specification fails to provide a representative number of species for the claimed genus to show that applicant was in possession of the claimed genus. A representative number of species means that the species, which are adequately described, are representative of the entire genus. Applicant also points to two publications, for example the Shaner et al. reference indicating that these reference represent two fluorescent proteins. However, these references represent post-filing data, thus not persuasive. As stated in the Office action mailed May 25, 2006, the MPEP states that ..." it is impermissible to use a later factual reference to determine whether the application is enabled or described as required under 35 U.S.C. 112, first paragraph..." (see, MPEP chapter 2100). Thus, for the reasons herein and stated above the rejection remains.

The arguments pertaining to the rejection under 35 U.S.C. 112, first paragraph enablement were considered, however are not persuasive. Applicant on pages 12-14 of the

amendment filed on July 12, 2006, addresses this rejection. Applicant state that the courts have clearly taught that the fact that experimentation may be complex does not necessarily make it undue, if the art typically engages in such experimentation. Applicant points to MPEP 2164.01 , citing the Federal Circuit for support of this statement. Applicant also points to Exhibit A, stating that guidance is provided with respect to conserved regions. This argument is not persuasive. With regard to the citation from *In re Certain Limited-Charge Cell Culture Microcarriers*, applicant's comments have been considered in full, however, the issue at hand is the breath of the claims in light of the predictability of the art as determined by the number of working examples, the skill level artisan and the guidance presented in the instant specification and the prior art of record. This make and test position is inconsistent with the decisions of *In re Fisher, 427 F.2d 833, 839, 166 USPQ 18, 24 (CCPA 1970)* where it is stated that "...scope of claims must bear a reasonable correlation to scope of enablement provided by the specification to persons of ordinary skill in the art...". Undue, experimentation would be required to practice the claimed invention because it is not routine in the art to test 68 $x10^{20}$ variants as is encompassed in the claims. While recombinant and mutagenesis techniques are known in the art, it is not routine in the art to screen large numbers of mutated proteins where the expectation of obtaining similar activity is unpredictable based on the instant disclosure. The amino acid sequence of a protein determines its structural and functional properties, and predictability of what mutations can be tolerated in a protein's sequence and result in certain activity. This is very complex, and well outside the realm of routine experimentation, because accurate predictions of a protein's function from mere sequence data are limited. Thus, the rejection remains for the reasons stated above and herein.

Conclusion

7. No claims are allowable.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Hope A. Robinson whose telephone number is 571-272-0957. The examiner can normally be reached on Monday-Friday.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Kathleen Kerr, can be reached at (571) 272-0931. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

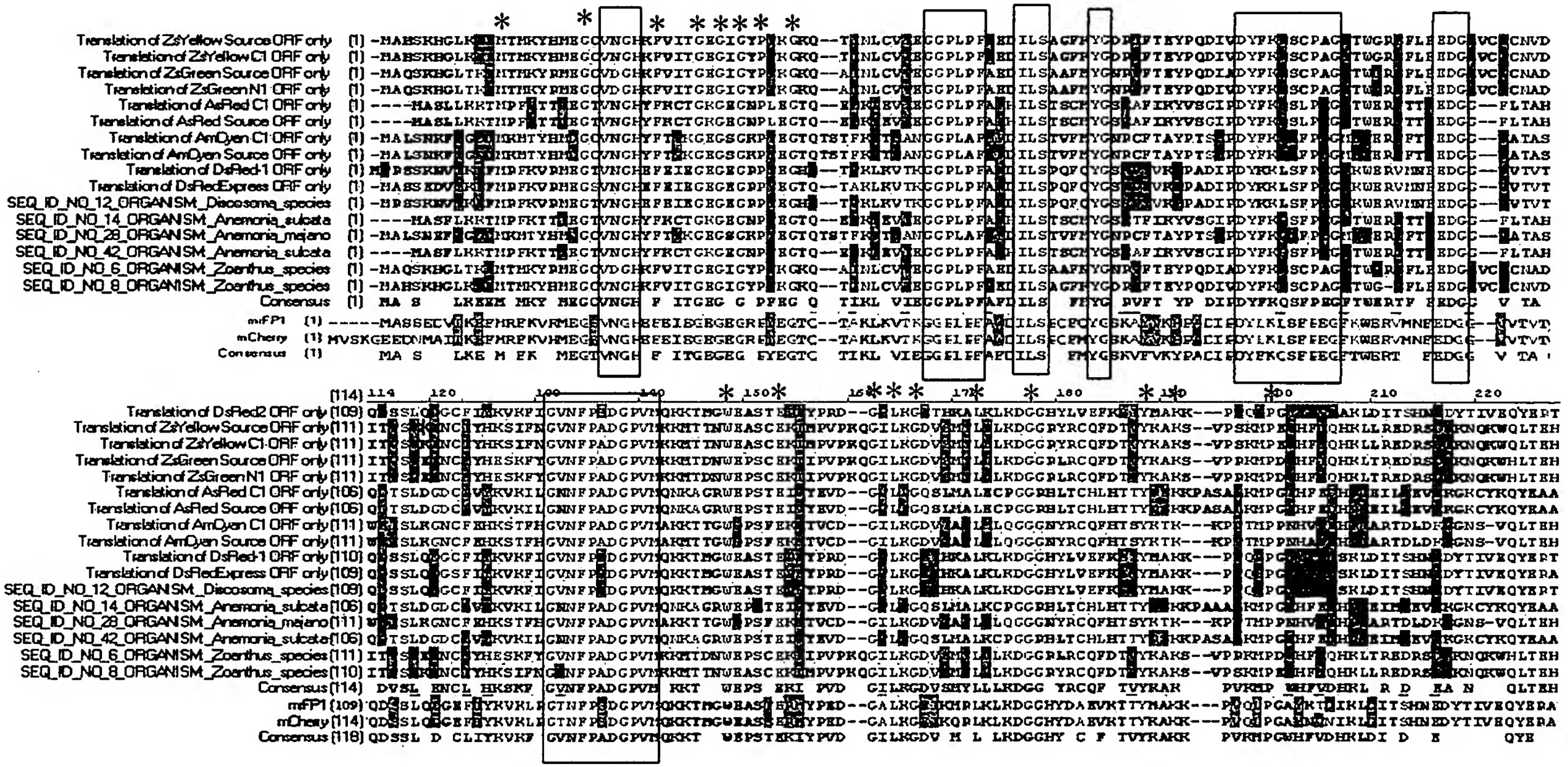
Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

Hope Robinson, MS

Patent Examiner 7/28/06

HOPE ROBINSON
PATENT EXAMINER

Exhibit A - Alignment of Chromo- or Fluorescent Proteins



Boxes and asterisks highlight regions and individual residues conserved in all proteins